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14. ABSTRACT

Based on our initial screening, we have identified a number of candidates that are involved in DNA damage repair pathway mediated by BRCA1, which is an important aspect of tumor suppression of the molecular. As none of the candidates have previously been reported to play a role in DNA damage repair pathway we speculate that some novel components, such as RNA or chromatin structure, might be involved. Once verified and fully investigated, the possible target genes will contribute to the understanding of the biology of inherited breast cancer as well as the diagnosis and treatment of breast, ovarian and possibly other cancers.

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Table of Contents

INTRODUCTION

BRCA1, as well as BRCA2, were identified as the hereditary breast and ovarian cancer susceptibility genes that can account for almost all the entirety of inherited cases of breast cancers (1-3). To study the biological function of the tumor suppressor BRCA1, we have proposed to perform genetic screening using a lentiviral vector based cDNA library which expresses 17,500 full length human and mouse genes. The library features a number of novelties over traditional ones, including broader target cell range, high efficiency, high quality, easier recovery of target genes and full length cDNAs. This methodology will not only uncover genes involved in BRCA1 tumor suppressor function but also lead to a general application to functional studies in cancer biology. The possible target genes isolated from the screens will contribute to the improvement of the understanding the biology of inherited breast cancer as well as the diagnosis and treatment of breast, ovarian and possibly other cancers. The identified genes will provide targets for the pharmaceutical industry to develop chemopreventive agents, a novel concept to our knowledge that will allow proactive intervention with the diagnosis of a BRCA1 mutation.

BODY

We have proposed to perform genetic screening using a lentiviral vector cDNA library to undertake the following three tasks:

1. **Cloning, identification, and characterization of genes that bypass BRCA1 deficiency mediated developmental arrest**
2. **Identification and cloning of gene(s) cooperating with BRCA1 to confer resistance to DNA damage**
3. **In vivo validation of BRCA1 second site suppressors.**

In the past year, we have made a significant progress in task 2 which will be described in detail below. As for task 1, we failed to isolate sufficient amount of BRCA1-/- MEFs due to the poor proliferation property of the cells. Thus, as proposed in the statement of work, we will have to perform the screen using mouse fibroblast derived from animals that have the endogenous BRCA1 floxed. We have successfully isolated such fibroblasts containing floxed allele spanning exon 5 to 13 (NCI Mouse Repository). We are constructing a modified self-deleting CRE lentiviral vector which contains a puromycin cassette downstream of the LoxP site in the U3 of the remnants of the LTR. Thus after expression of CRE and deletion of the endogenous floxed BRCA1 allele and the CRE expressing vector, it will still leave a puromycin expressing cassette integrated in the genome.

BRCA1 deficient HCC1937 cells are unusually sensitive to DNA damage induced by gamma irradiation. We exploited this sensitivity to screen for dominant suppressors of this phenotype by introduction of a lentiviral library containing 17,500 full length human cDNAs as described in the main body of the grant. In our experience in the irradiation of this cell line we typically irradiate 10,000 to 50,000 cells with a dose of 4.5 Gray. At this dose the cells typically do not survive or form not more than 2 or 3 colonies as an upper limit. BRCA1 reconstituted cells typically form around 20 to 40 colonies under these identical conditions. This indicates that even a full reversal of the BRCA1 defect only leads to approximately to the survival of one out

of a 1000 reconstituted cells. When using a library only single or a few cells are expected to be transduced with any given cDNA, thus irradiation under the same conditions would most likely miss a gene that might rescue or ameliorate the phenotype. Thus we designed a screen based on many rounds of enrichment of lower sublethal doses of radiation that should give selective growth advantage to those genes that had a positive effect on proliferation in the presence of DNA damage (Fig. 1).

To accomplish this we irradiated HCC1937 cells with incremental doses ranging from 1.0 Gray to 3.0 Gray allowing 48 hours for recovery. In the present study we performed seven rounds of such irradiations. After the final round of irradiation we pooled all the surviving cells and extracted DNA from the pooled population. By PCR amplification using T3 and T7 primers, which flank the cDNA insert, we identified 6 distinct bands ranging in size from approximately 0.7 kbp to 2.8 kbp. (Figure2). Control HCC1937 cells that were not infected with the library yielded no products.

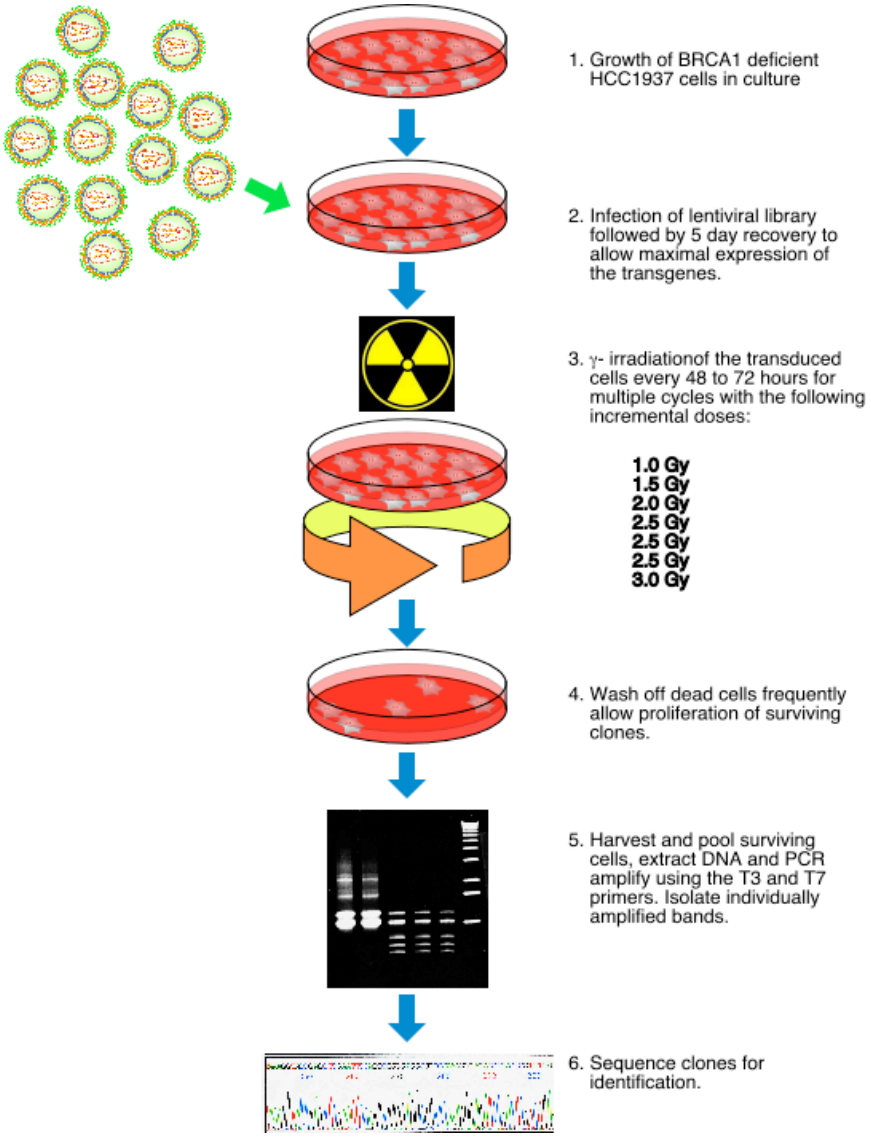

Figure 1. Scheme of the lentiviral library screening.

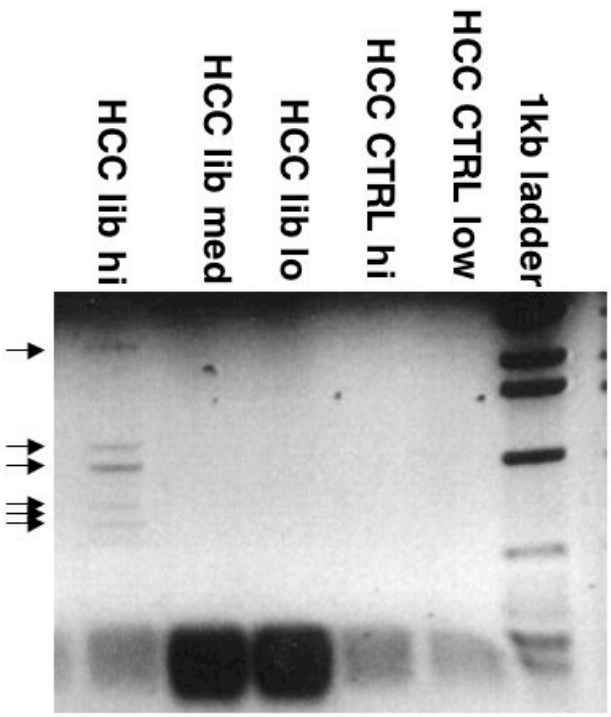

Figure 2. The PCR products of the pooled surviving cells. PCR products had to be reamplified for sequencing purposes. Sequencing of these clones identified the following genes (Table1).

Table 1. Summary of clones identified after gamma irradiation selection

Clone	Identity	Accession #	Description
5-1	Unknown protein	BC009103	Novel ORF overlapping COX4
5-2	Tumor suppressor	AF156165	Putative tumor suppressor in -5q syndrome
5-4	L36A-like protein	NM_001001	Similar to human Ribosomal protein L36
5-5	PKCBP1	BC060508	Protein Kinase C binding protein 1
5-6	H3.3B	BC067757	Histone variant H3.3 B

To validate the candidates, we have transfected clones 5-1, 5-4, 5-5, and 5-6 into an expression lentiviral vector, pBOB-CAG. We will individually transduce them into HCC1937 cells and subject the cells to irradiation and observe the resistance of these cells. Once the clones are validated, we will further characterize the mechanism of these clones in substituting BRCA1 in conferring resistance to DNA damage. Obviously, all clones are novel factors that have not been reported in DNA damage repair pathways. We did not identify BRCA1 as a positive clone can be explained by the absence of this gene in the cDNA library.

KEY RESEARCH ACCOMPLISHMENTS

1. Established mouse fibroblasts harboring floxed BRCA1 allele spanning exons 5 to 13.
2. Identified and cloned four novel factors cooperating with BRCA1 to confer resistance to DNA damage.

REPORTABLE OUTCOME

1. Established mouse fibroblasts harboring floxed BRCA1 allele spanning exons 5 to 13.

CONCLUSION

Based on our initial screening, we have identified a number of candidates that are involved in DNA damage repair pathway mediated by BRCA1, which is an important aspect of tumor suppression of the molecular. As none of the candidates have previously been reported to play a role in DNA damage repair pathway we speculate that some novel components, such as RNA or chromatin structure, might be involved. Once verified and fully investigated, the possible target genes will contribute to the understanding of the biology of inherited breast cancer as well as the diagnosis and treatment of breast, ovarian and possibly other cancers.

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